CHARGE TO REVIEWERS: INTERLABORATORY STUDY OF WHOLE EFFLUENT TOXICITY TEST METHODS

The U.S. Environmental Protection Agency (EPA) is conducting a peer review of the scientific "Study Plan for Determining Interlaboratory Variability of the EPA Short-Term Chronic and Acute Whole Effluent Toxicity (WET) Test Methods." The overall goal of the peer review is to enhance the quality and credibility of Agency decisions by ensuring that the scientific and technical work products underlying these decisions receive appropriate levels of peer review by independent scientific and technical experts. This charge to the reviewers was developed in accordance with EPA's established peer review guidelines and policies and is meant to ensure that the Agency uses credible and appropriate science in the evaluation of each test method for the whole effluent toxicity testing program (EPA, 1998). The Interlaboratory Study design (Appendix A) outlines the procedures for the testing, sample handling, selection of laboratories, and general information on the conduct of the toxicity tests to be evaluated. At least nine laboratories will be conducting tests to evaluate the performance of twelve of the seventeen promulgated WET test protocols. The goals of the interlaboratory study are to evaluate the variability of observed results when different analysts conduct tests on the same samples (i.e., the precision of the test methods), to determine the rate at which participating laboratories successfully completed tests initiated, and to evaluate the rate at which the tests indicate "toxicity" is present when measuring non-toxic samples. EPA will evaluate the study results to draw conclusions about the performance of standardized WET tests.

The charge to the Peer Review Panel is to objectively review whether the Interlaboratory Study design (Appendix A) effectively assesses method precision, test completion rate, and the rate at which the tests indicate "toxicity" when measuring non-toxic samples. This charge to reviewers, the study design and, ultimately, the study results will be available in a public docket. Public comments received in the docket will be available for review upon request.

Background:

EPA's WET testing policies and regulations are intended to support goals of the Clean Water Act, specifically, to provide for the protection and propagation of fish, shellfish, and wildlife. The Clean Water Act (section 101(a)(3)) states that "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited." Through the water quality standards program and the National Pollution Discharge Elimination System (NPDES) permitting program, this objective has been pursued. A major step forward for toxics control was the adoption of water quality-based permitting to integrate chemical and biological monitoring to protect receiving water quality. The integration of the effluent effects and receiving water exposure measurements resulted in the development of effluent hazard assessment approaches.

Acute and short-term chronic WET tests estimate the toxicity of wastewaters in order to protect aquatic life. These tests measure the aggregate toxic effects of an effluent to standardized, freshwater or marine plants, vertebrates or invertebrates. The standardized tests are used for both monitoring effluents and receiving waters, and for NPDES water quality-based permit limits. When technology-based permit limits are insufficient to protect water quality, a permit will

include an effluent limitation for WET if the discharge would cause, have a reasonable potential to cause, or contribute to an instream excursion above a numeric water quality standard for WET or a narrative water quality standard (e.g., a standard to prevent the discharge of toxic pollutants in toxic amounts). The results of a single test could be used to assess compliance with a permit limit for WET (expressed in terms of acute or chronic toxic units). For example, an end-of-pipe permit limit of 0.3 TU_a may be established when the State water quality standards have no mixing zone allowance, or an end-of pipe permit limit of 1 TU_c may be established when there is little or no dilution instream.

Currently, the Agency does not consider method variability when calculating permit limits. The Agency is in the process of developing variability guidance and the results of the study may become part of the database cited in that guidance. A further explanation for WET in the regulatory arena is listed in Chapter 3 of the *Technical Support Document for Water Quality-based Toxics Control* (EPA, 1991).

Intention Behind the Study Design:

The three primary goals of this interlaboratory testing are to determine the precision associated with several WET test protocols in their current form, as well as the test completion rate and the rate at which the tests indicate "toxicity" when measuring non-toxic samples. By using the term *precision*, the Agency means to describe the measurement of mutual agreement among individual measurements of the same property by using sample data generated from replicate measurements. The *test completion rate* will be based on the number of laboratories that initiate testing, complete the tests, and meet test acceptability criteria. The rate at which the tests indicate "toxicity" when measuring non-toxic samples will be assessed by evaluating the completed tests that indicate toxicity in reagent water samples, also known as blanks.

To describe precision, these interlaboratory studies will assess repeatability variance (based on *intralaboratory* data) and reproducibility variance (based on *interlaboratory* data) and evaluate between-analyst variance. By using the term *repeatability*, EPA intends to describe the variability that arises between tests using a single test protocol conducted within a single laboratory with a single operator conducting the testing. With repeatability held reasonably constant (same individual, same equipment) the variability should be low (ASTM, 1992). By using the term *reproducibility*, EPA intends to describe the variability in test results expected between different analysts using different equipment in different laboratories. Reproducibility would incorporate variability introduced by the differences among laboratories, such as the equipment used, operators, calibration of equipment, and laboratory conditions. Interlaboratory variance may be dependent upon how a variety of laboratories follow a specific test protocol. EPA has designed these interlaboratory studies to require adherence to specified protocols, a demonstration of qualifications and experience, a demonstration of quality control practices and performance, and in these studies, a demonstration of performance on a set of samples. In designing these studies, EPA consulted an interlaboratory study guidance for determining the precision of a test method developed by the American Society of Testing and Materials (ASTM, 1992).

Interlaboratory Study Overview:

During 1998 and 1999, EPA will conduct the interlaboratory variability studies to assess the method precision of the toxicity test methods listed below. It is EPA's intent to quantify interlaboratory variability and estimate precision for each test endpoint in the study. The study is also designed to provide data on the success rate for test initiation and completion for each test method and the frequency of toxicity detection.

To evaluate each of the twelve methods, a minimum of nine laboratories will analyze test samples provided by an EPA contractor. The laboratories selected by EPA should typify laboratories that routinely conduct WET testing for permittees. The study is designed to maximize the number of qualified laboratories that can participate. Laboratories must meet prequalification requirements, including but not limited to: historical records of acceptable control charts using reference toxicants, documented experience in using appropriate test conditions, ability to meet test acceptability criteria, and proficiency in the application of appropriate statistical analyses for each test and test endpoint for each method that the laboratory will perform in the interlaboratory study. The study design is provided in Appendix A.

EPA will provide specific instructions to participating laboratories that they are to diligently follow the specific WET test procedures as promulgated. These instructions will also highlight method-specific requirements included in the OST policy memorandum regarding WET test methods flexibility and the study plan to evaluate certain aspects of specific tests (e.g, Ceriodaphnia reproduction test intervals, Selanastrum test and the effect of EDTA) (EPA, 1996). All data collected by each participating laboratory during the course of the testing must be reported, including data from tests that were not completed for any circumstance. The following specific WET methods will be evaluated for the interlaboratory study:

From the Freshwater Chronic Toxicity Manual (3rd edition)
Method 1000: *Pimephales promelas* (fathead minnow) Larval Survival and Growth Test
Method 1002: *Ceriodaphnia dubia* (cladoceran) Survival and Reproduction Test
Method 1003: *Selenastrum capricornutum* (green alga) Growth Test (with and without EDTA)

From the Marine Chronic Toxicity Manual (2nd edition)
Method 1004: *Cyprinodon variegatus* (sheepshead minnow), Larval Survival and Growth Test
Method 1006: *Menidia beryllina* (inland silverside), Larval Survival and Growth Test
Method 1007: *Mysidopsis bahia* (mysid shrimp), Survival, Growth, and Fecundity Test
Method 1009: *Champia parvula* (red macroalga), Reproduction Test

From the Acute Toxicity Manual (4th edition)
Ceriodaphnia dubia
Pimephales promelas
Cyprinodon variegatus

Menidia beryllina
Holmesimysis costata (using the test procedures to measure acute toxicity on *Mysidopsis bahia*).

Questions for Peer Reviewers:

To facilitate review of the study design, the four questions below should be considered. Note that the review is primarily concerned with an evaluation of the study design in terms of its ability to assess test method precision, test completion rate, and the rate at which the tests indicate "toxicity" when measuring non-toxic samples, not the application or implementation of the whole effluent toxicity control requirements generally. The primary function of the peer reviewer should be to judge whether the study design and interpretation of the data can provide meaningful and useful estimates of method performance.

1) Evaluate the conceptual soundness of the approach, study plan, and anticipated study results for obtaining estimates of precision, test completion rate, and the rate at which the tests indicate "toxicity" when measuring non-toxic samples. Is the approach clear and is it appropriate? Are there additional parameters that should be included in the testing program that are not apparent in the study design?

2) Are the number of sample replications and participating laboratories appropriate to evaluate the precision, test completion rate, and the rate at which the tests indicate "toxicity" when measuring non-toxic samples of the methods in actual use?

3) Are the laboratory prequalification conditions and procedures appropriate to ensure that the study will be performed by laboratories representative of those that routinely conduct WET testing for permittees throughout the United States?

4) Within the context of the intended regulatory use, is the interlaboratory study appropriately designed to gather scientifically acceptable information on test precision, test completion rate, and the rate at which the tests indicate "toxicity" when measuring non-toxic samples?

Time Frame for Review:

EPA requests comments on the interlaboratory study proposal from the peer review panel by NO LATER THAN DECEMBER 9, 1998. Following the peer review period and consideration of comments, EPA anticipates that the study will begin in March 1999. EPA plans to submit the results of the interlaboratory validation studies for peer review on or before February 24, 2000.

Copies of the public comments received and all other supporting documents (including references included in this charge to peer reviewers and Appendix A) will be available for review on Monday, August 24, 1998, at the U.S. Environmental Protection Agency, Water Docket, 401 M Street SW, Washington, DC 20460. Peer reviewers and the public may contact the Water Docket at (202) 260-3027 on Monday through Friday, excluding Federal holidays, between 9:00 am and 3:30 pm Eastern Time for an appointment.

References:

ASTM, 1992. *Standard Practice of Conducting and Interlaboratory Study to Determine the Precision of a Test Method*. ASTM 14.02, E691-92. American Society for Testing and Materials, West Conshohocken, PA.

EPA, 1998. *Science Policy Council Handbook: Peer Review*. EPA Office of Science Policy and Office of Research and Development, Washington, DC. EPA 100-B-98-001.

EPA, 1996. *Policy Memorandum: Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods - April 10, 1996*. Office of Science and Technology, Washington, DC.

EPA, 1991. *Technical Support Document for Water Quality-based Toxics Control*. Office of Water, Washington, DC. EPA-505-2-90-001.